Measles Vaccination and Nutritional Status among Hospitalised Infants and Children in Rural Tanzania; A Field Study

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Short title:

Measles and nutrition among Tanzanian children

Summary

A large number of children in developing countries contract and die from acute measles infection each year despite various strategies for measles prevention through vaccination programmes. In a rural area in the central part of the Republic of Tanzania, East Africa, all children admitted to a local hospital were investigated for anamnestic information of previous vaccination against measles and for information of previous measles disease. Sera from the children were examined for levels of anti-measles antibodies and for fat soluble vitamins (vitamins A, D and E). Among previously healthy, vaccinated children 71.9% had detectable antibodies to measles virus and parents' information also indicated higher levels of protection against clinical measles among vaccinated compared to unvaccinated children. Serum levels of measles antibodies correlated with the number of measles vaccinations received. The children were generally malnourished and showed low levels of vitamins A and E, but normal levels of vitamin D. In conclusion, routine vaccination under field conditions yields protection against measles but emphasis on both improved vaccination coverage and nutritional status must be implemented in order to eliminate measles in developing countries.

Introduction

Measles is still of major importance in terms of child morbidity and mortality world-wide. Each year approximately 45 million infants and children under five years of age develop measles and of these about one million die as a result of the disease. In order to eliminate measles it has been assumed that at least 95% of susceptible children must receive a vaccine that is 100% effective[1]. This has proven difficult and the World Health Organisation (WHO) has thus defined a 90% reduction of total measles cases and a total reduction of 95% in mortality, compared to prevaccination levels, as a goal[2].

Severe measles is still a problem confined mostly to developing countries and in several African countries including Tanzania, measles epidemics still occur in spite of vaccination campaigns[3,4]. In some of these countries, the case fatality rate (CFR) of acute measles disease has previously been reported very high[5] but both mortality and incidence have declined after the introduction of measles vaccination[1,6]. In contrast, an increase in CFR has been reported in countries where vaccination campaigns at least periodically, have had a lower priority[7].

Previous reports have estimated a vaccine efficacy in African countries of less than 60%[8]. Measles epidemics within vaccinated populations in these countries may be attributed to the use of inactive vaccines (i.e. due to cold-chain breaks), presence of maternal neutralising antibodies (if child is vaccinated before one year of age) and also to insufficient vaccine coverage (less than 95%[2]).

All degrees of malnutrition have been observed both in the acute phase of measles infection and also as a long term complication[9]. Vitamin A deficiency has been documented as a major cofactor of morbidity and mortality in children as a result of impaired cellular immunity due to changes in T-cell function[10]. There is also evidence that

measles infection depresses the levels of serum vitamin A[11], and recent meta-analyses have proven that the introduction of early vitamin A supplements improves survival in diseased children[12,13].

In the present study we wanted to describe the relationship between measles vaccination status under routine field conditions, and the levels of anti-measles antibodies among children and infants in a rural area of Tanzania who were hospitalised for other diseases than measles.

Based on our findings, we suggest that in this area of Tanzania, measles may be eliminated by enhanced vaccination efforts, primarily by increasing vaccination coverage. Improved nutritional status by increased vitamin A intake may also prove beneficial.

Materials and Methods

Patient selection

All children and infants, aged between nine months and five years admitted to Haydom Lutheran Hospital during a four-month period (from June to September) were included in the study (n=243 of which 133 were male, 104 female and 7 of unreported sex). The total number of people estimated to live in the Haydom recruitment area exceeds 300 000. The study was approved by the Tanzanian Commission for Science and Technology (Permission CST/RSA.91/160/839/92). When a blood sample was taken, the child's parents were informed and gave consent.

Clinical examination

The children were subjected to clinical examination by the hospital's Medical Assistants (MAs) at the time of admission. The MAs are local health personnel with a two-year practical medical education that focuses on the most common health problems in Tanzania and were therefore found qualified to evaluate the children clinically. A special registration form was designed for registration of past measles history, current clinical diagnosis, nutritional status and number of vaccinations against measles. The MAs were supervised on how to fill out the registration forms, and their work was controlled daily during the first two weeks of the study.

If the parents gave anamnestic information about a previous history of a typical rash in combination with fever, cough, coryza and conjunctivitis, the child was classified as having suffered from measles.

Nutritional status

The best indicator for child nutrition is most likely weight for height. However, weight for age in percent of reference weight median has been used earlier for comparison of mortality

risk in undernourished African children and found useful under field conditions[14]. The nutritional status (termed "weight index") in this study, represent the child's relative weight in comparison to median WHO standard for the corresponding age group[15].

Serological examination

As soon as possible after admission a blood sample was obtained by venipuncture. The samples were centrifuged and the serum was transferred to cryotubes for storage at -20°C before transportation to the University of Bergen, Norway where further analyses were performed. When a serum sample had not been taken, usually due to early discharge from the hospital, notes on the children's clinical status were kept as a reference material for nutritional status and previous illnesses.

Detection of antibodies against measles virus

Anti-measles IgG were quantified by Enzyme Linked Immuno Sorbent Assay (ELISA), (Enzygnost® for Measles, Behringwerke AG, Marburg, Germany), according to instructions provided by the manufacturer. The optical density (OD) values were detected by an automated ELISA-reader (Titertek® Mulitiscan PLUS, Labsystem, Finland). The background in the virus negative control well was subtracted from the obtained OD value at 405 nm (giving the ΔOD). The negative cut-off value was defined as a ΔOD less than 0.2. For calculations of individual antibody levels, the ΔOD values were used directly, as these values demonstrated linear relation with the ODs obtained with a two-fold dilution of a known positive serum (not shown).

In some sera where the negative (non-specific) background OD values were more than twice the value of defined OD for a positive serum (background > 0.4), a supplementary ELISA assay was performed (SIA, Sigma Diagnostics, St. Louis, MO, USA) according to instructions provided by the manufacturer.

Detection of fat soluble vitamins (vitamins A, D and E)

Serum samples from fifty children from whom enough serum was available for analysis were used to detect fat soluble vitamins. Serum 25-hydroxyvitamin D (OHD) was determined by a competitive protein binding assay after extraction of 100 µl serum with chloroform/methanol (2:1, v/v) and purification on open silicic acid columns[16].

Retinol (vitamin A) and α-tocopherol (vitamin E) were determined by a modification of a previously described method[17]. Fifty µl serum and 50 µl of an ethanolic solution of retinylacetate and α-tocopherol-acetate were extracted by 1.5 ml hexane. The hexane layer was evaporated to dryness by a stream of N_2 and redissolved in 125 µl methanol and injected to a HPLC system using a 5 µm C-18 column and methanol/water (96:4, v/v) at a flow rate of 2.5 ml/min. The eluate was monitored by a UV-detector at 295 nm.

Reference values describes vitamin levels above values giving rise to clinical signs of vitamin deficiency[18-20].

Of the 50 children in which vitamin levels were quantified, 48 and 42 children, respectively, could also provide information regarding age and weight index.

Statistics

Comparisons of single means between groups were performed by student's t-test. Comparison between proportion of groups were performed by Fisher's exact two-sided test for non-parametric measures. Results were regarded as significant when $p<0.05$.

Results

Measles antibodies and vaccination

A total of 223 children could provide data regarding their vaccination status. 85.2% (n=190) of these children were reported to be vaccinated against measles. Sera from 168 children were tested for antibodies against measles virus. Of these, 156 children could provide more information of their child's vaccination record. All results regarding vaccination status and previous measles history are summarised in Table 1. Among the entire group of tested and untested children, a significantly lower fraction of a previous history of clinical measles was found in the group of children who had been vaccinated compared to the unvaccinated group (5.5% for n=165 vs. 13.8% for n=29, respectively, $p<0.001$). Anti-measles antibodies were detected among a significantly higher proportion of vaccinated children than among children with no anamnestic information of measles vaccination (69.2% for n=130 vs. 38.5% for n=26, respectively, $p=0.006$).

When antibody levels were determined by ΔOD in the Enzygnost® assay, significantly higher antibody levels was observed in the group receiving two doses compared to the group having received one dose of measles vaccine ($p=0.02$, Figure 1). Significantly higher antibody levels were found in both of the vaccinated groups when compared to the unvaccinated group ($p=0.04$ and $p<0.004$ for one vs. zero doses and two vs. zero doses, respectively).

Using the supplementary testing kit for detection of measles antibodies on sera with a high non-specific background OD, the fraction of anti-measles antibody positive sera in the group of vaccinated children without a history of previous measles increased. However, the analysis was done on a very limited number of sera and the results were thus omitted from further statistical analysis.

Weight index

A large fraction of the children was defined as being malnourished (Figure 2) when nutritional status was evaluated on the basis of weight by age in percent of reference median[15]. The weight index varied widely irrespective of age and clinical diagnoses at admission, of which malaria, respiratory tract infections and gastro-enteritis were the most prevalent (data not shown). Although the number of children was low, a significantly higher weight index was found in infants and children attending the MCH clinics, as opposed to those who did not (mean scores of 91 and 68 respectively, $p<0.05$). In accordance with previous investigations reporting long-term malnutrition after measles[9], a lower weight index was observed among children who had suffered from the disease prior to hospitalisation with mean weight indexes of 72.3 and 79.9 in the two groups ($p<0.05$)

Vitamins and nutrition

As the introduction of vitamin A supplements has increased survival in acute measles infection[14,15], we measured levels of this vitamin, as well as that of other fat soluble vitamins in a random selection of sera from 50 children. A correlation between serum vitamin A levels and the weight index was documented ($r=0.34$, $p=0.028$) (Figure 4a). We also found a significant negative correlation between vitamin D levels and age ($r=-0.43$, $p=0.002$). Serum concentrations of vitamin A and E were below levels associated with clinical signs of vitamin deficiency in 21% and 54% of the children respectively[18,20], whereas normal values of vitamin D were observed[19]. We found no correlation between vitamin A levels and the children's ages or measles histories (data not shown).

Discussion

In this study, we found that it is possible to obtain a relatively high rate (85%) of vaccine coverage under routine conditions among children living in a very rural district in central Tanzania. Although the vaccination coverage in this study is higher than what could be expected, it is clearly below the recommended 95% coverage necessary for measles eradication[2]. Vaccine coverage must thus be improved as a first step to reach the WHO goal. Although only 69.2% of the vaccinated children had detectable antibodies against measles, the vaccinated children in our study reported a lower frequency of previous measles. This suggests that the local vaccine regimen potentially could induce a satisfactory level of disease protection among immunised children, implying that once a higher coverage is achieved, measles control may be attained in this area.

The vaccination status of each child was obtained by questioning the child's parents. Herein lies obvious bias of over-reporting of vaccination coverage, and a corresponding under-reporting of actual seroconversion to the vaccine[21]. However, since the study took place in a district in which a large portion of the population belongs to a nomadic tribe, the probability of having the children bringing their MCH card to hospital was considered to be low. For all practical purposes, parents' recall was therefore accepted for determining vaccination status and measuring disease prevention under field conditions.

Interestingly, the parents information was supported by the serological data both in terms of the fraction of children having detectable antibody levels (Table 1) and by demonstrating a relationship between the number of vaccinations received and the levels of anti-measles antibodies (Figure 1).

In developing countries where children get measles at a very young age it is important to immunise them as early as possible after they have lost the passive protection mediated by

maternal antibodies. Since the antibodies disappear at different ages in the individual children, a two-dose vaccine regimen has been recommended[22]. Our results which show higher levels of antibodies in sera from children having received two doses compared to one, speak in favour of a two-dose strategy using the vaccine strain presently available.

A surprisingly high proportion (38.5%) of the unvaccinated children without any reports of previous measles had detectable antibodies in their sera. However, mean ΔOD for these children as a group, was below the defined cut-off level in the Enzygnost® assay. Nevertheless, some of them may have had measles without it being recognised.

Since children admitted to hospital have previously been used to reflect measles dynamics in rural areas in Africa[23], we also assumed that the children in our material did not differ significantly from other children in the area and that they therefore may reflect the general health situation in the area from which the hospital recruits patients. In this study malnourishment was widespread without any difference between the sexes (Figure 2). The children observed are most likely representative of their kin also in terms of weight by age, thus indicating that the general nutritional level in the area is below recommended standards.

The best indicator for child nutrition is most likely weight for height. However, weight for age in percent of reference weight median has been used earlier for comparison of death risk in undernourished children and found useful under field conditions[14].

Our data, although limited, demonstrate a significantly impaired nutritional status in the infants and children who have suffered from measles. As shown in Figure 3, there was also a significant relationship between general nutritional status and serum vitamin A levels. The low levels of serum vitamin A and E could be explained be the overall malnourishment observed by the weight-scores as the levels of these vitamins rely on dietary intake in contrast to vitamin D that increase upon exposure to sunlight and thus

were normal in most children included in this material. It is an established fact that vitamin A supplements reduces mortality from acute measles[24]. The children in our study who attended an MCH clinic had a higher weight index than children who had not been in contact with these institutions, strongly emphasising the impact of MCH clinic work. Better MCH-clinic attendance could therefore be an important tool for increasing general nutritional status among children in the area and thus be a factor for inducing higher vitamin A levels. In a broader sense it would be important to improve dietary intake of vitamin A in areas where children are prone to suffer from potentially chronic or deadly viral diseases.

In conclusion our study indicates that vaccination against measles may be successful in a developing country, such as Tanzania, if certain prerequisites are met. If the recommended vaccine coverage of 95% and a two-dose regimen could be obtained along with higher attendance to the MCH-clinics, eradication of measles is within reach. To evaluate the effect of measles vaccination in this area, further epidemiological surveys are warranted and are in progress.

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Table 1

Measles vaccination status for 243 infants and children from rural Tanzania. The group of children classified as vaccinated represents the sum of children with a definite history of vaccination and children likely to have been vaccinated due to previous visits to MCH clinics when they were at the right age for vaccination. The group classified as unvaccinated represents the sum of children who were reported as definitely not vaccinated and children who had never attended an MCH clinic.

		Past measles			Measles antibodies		
	n	Yes	No	ND.[a]	Yes	No	NT.[b]
Vaccinated	190	9	156	25	90	40	60
Unvaccinated	33	4	25	4	10	16	7
No vaccine data	20	2	11	7	3	9	8
Sum	243	15	192	36	103	65	75

a) ND. Not determined due to inconclusive anamnestic information from parents of previous measles in the child. b) NT. Not tested for antibodies against measles virus due to lack of serum.

Figure legends.

Figure 1.

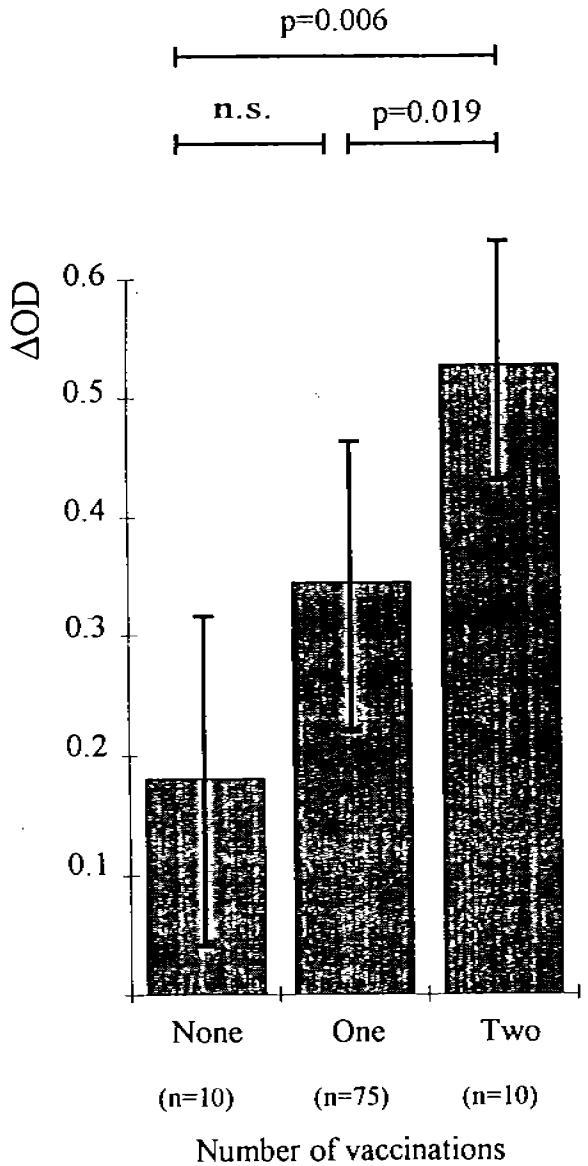
Levels of antibodies against measles virus presented as ΔOD values in the Enzygnost ELISA assay in sera from children without anamnestic information of measles disease. The number of individuals in each group is indicated in parentheses. Standard errors are included and p values indicates significance of differences between means (Student's t-test).

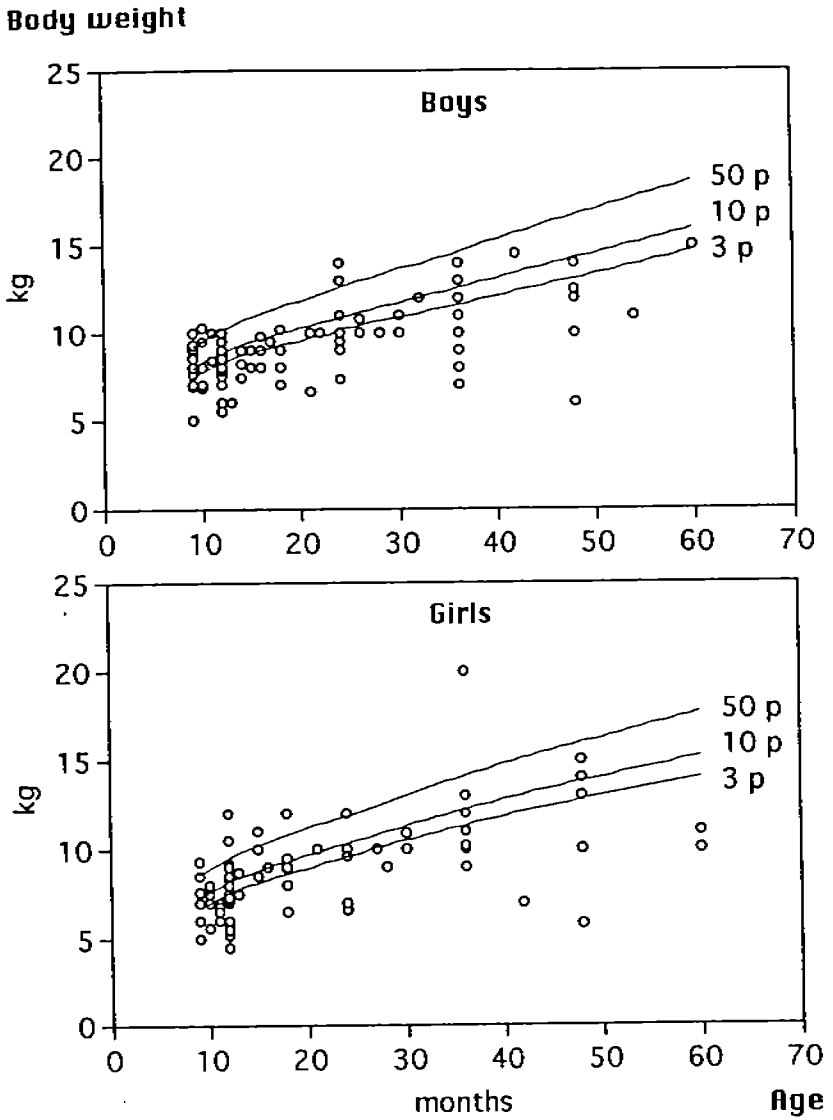
Figure 2.

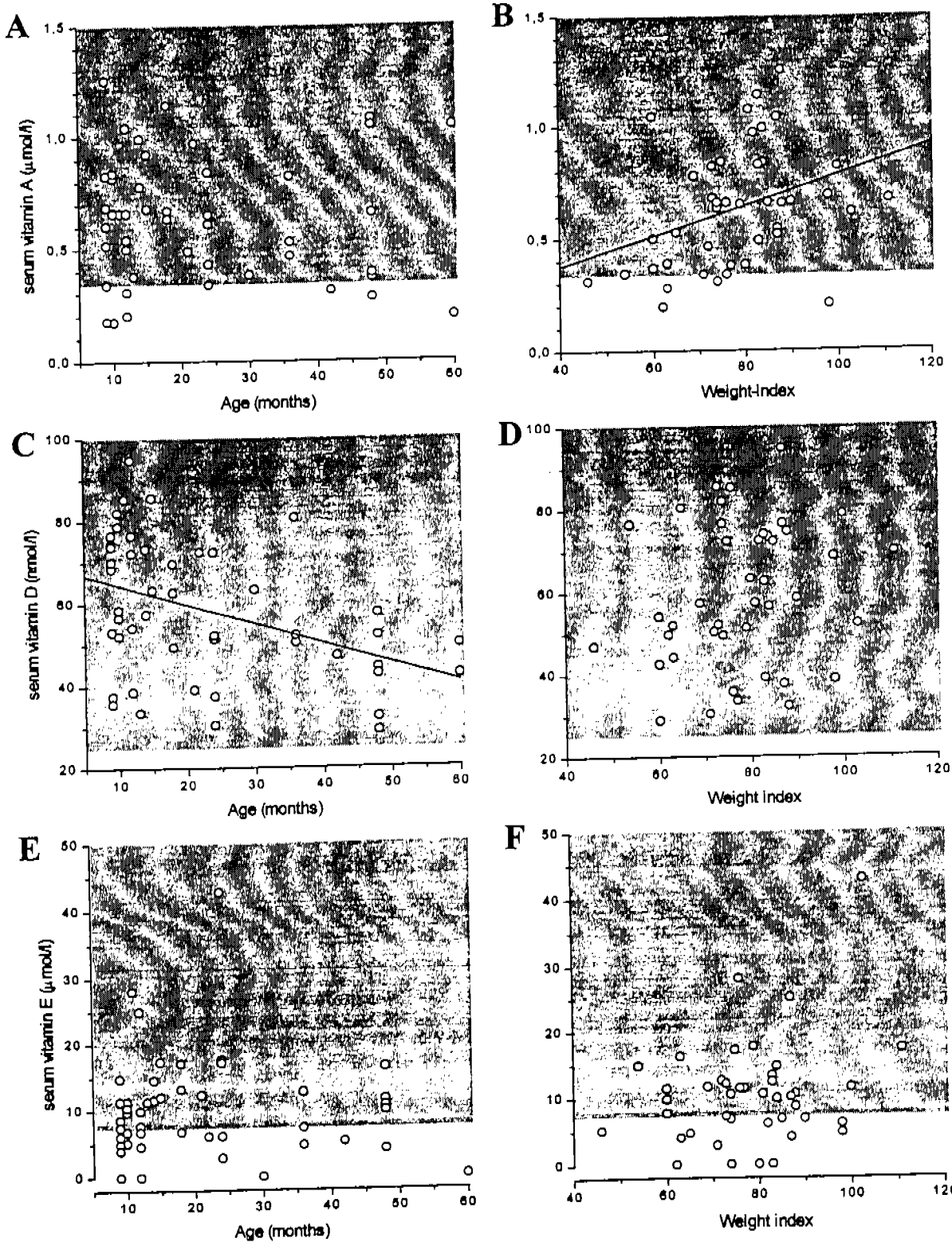
Body weights related to age among boys and girls. Lines are representing the 3rd, 10th and 50th percentile of reference weights of children[15].

Figure 3.

Levels of the fat soluble vitamins A, D and E in sera from 50 children in relation to the weight index (percent of reference median) and age. Shaded areas refer to normal values (representing normal range of serum vitamin levels in children without clinical signs of vitamin deficiency[18-20]).


p=0.006
n.s.
p=0.019
ΔOD
0.6
0.5
0.4
0.3
0.2
0.1
None
One
Two
(n=10)
(n=75)
(n=10)
Number of vaccinations


Body weight
Boys
Girls
kg
25
20
15
10
5
0
50 p
10 p
3 p
0
10
20
30
40
50
60
70
months
Age


A
serum vitamin A (µmol/l)
Age (months)
B
Weight-Index
C
serum vitamin D (nmol/l)
Age (months)
D
Weight Index
E
serum vitamin E (µmol/l)
Age (months)
F
Weight Index